

REMARKS

Rejection of Claims 40-76 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 40-76 under 35 U.S.C. § 112, first paragraph, on the basis of enablement. Specifically, the Examiner contends that until one knows the identity of the specific mutations in a glucosamine-6-phosphate synthase that account for increased enzyme activity or decreased product inhibition, then the specification simply extends an invitation to the skilled artisan to perform trial and error experimentation. The Examiner asserts that while the Declaration of Dr. Deng shows that wild-type genes from other biological sources may be used in the claimed method, neither the Declaration or the specification teaches how to make any microorganism comprising any genetic modification that increases glucosamine-6-phosphate synthase activity or any genetic modification in a coding region of glucosamine-6-phosphate synthase. The Examiner asserts that Example 5 shows experimentation that is outside the realm of routine experimentation to identify 6 microorganisms that are superior glucosamine producers. The Examiner contends that general teachings of screening and searching is not guidance for making and using the invention, and that such methods do not exclude, for example, site-directed mutagenesis on the coding sequence to screen and search for the recited mutations.

Applicants traverse the rejection of Claims 40-76 under 35 U.S.C. § 112, first paragraph. Initially, it is noted that the Examiner acknowledges that wild-type genes from other biological sources (*B. subtilis*, *C. albicans*, *S. cerevisiae*) can be used in the claimed method. However, the Examiner does not acknowledge enablement for Claim 71, for example, nor for part (a) of any of Claims 40, 70 or 72, where there is no requirement for any genetic modification other than overexpression of the nucleic acid molecule encoding glucosamine-6-phosphate synthase. Applicants have previously argued and provided evidence that, at the time of the present invention, genes encoding glucosamine-6-phosphate synthase, also known as glucosamine:fructose-6-phosphate amidotransferase, had been identified in many different organisms. As the Examiner acknowledges, Applicants have demonstrated that glucosamine-6-phosphate synthases from sources other than *E. coli* can be used in the claimed method. It is noted that these synthases all work in the claimed method *despite* lower overall sequence homology to the *E. coli* glucosamine-6-phosphate synthase,

illustrating that as long as the sequence encodes a protein with glucosamine-6-phosphate synthase activity, it can be predicted to operate in the claimed method to produce the glucosamine-6-phosphate product. Indeed, the fact that an enzyme is a glucosamine-6-phosphate synthase indicates that it is an enzyme that catalyzes the production of glucosamine-6-phosphate. Since multiple examples of this enzyme were known in the art at the time of the invention, Applicants submit that the claimed method which uses this enzyme is enabled, given the guidance provided in the specification for doing so.

With respect to the Examiner's assertion that it would require undue experimentation to make and use the glucosamine-6-phosphate synthases with the recited genetic modifications, Applicants submit that the Examiner is incorrect in his assertion that Example 5 shows experimentation that is outside the realm of routine experimentation to identify 6 microorganisms that are superior glucosamine producers. To the contrary, as Applicants have previously argued, it is not necessary to know where to modify the sequence in order to produce the recited microorganisms and use them in the claimed method. The Examiner has maintained his position that routine experimentation does not include making a vast number of mutants and screening and selecting those that have the desired phenotype of increased glucosamine-6-phosphate synthase activity. However, this line of rationale is completely contrary to the established practices of those of skill in the art of microbial strain improvement and fermentation biology, and indeed, the Examiner has provided *no evidence* that the use of the combination of molecular and classical techniques to produce, screen and select *multiple* microorganisms having the recited genetic modifications is anything but routine, particularly given the guidance provided in the present specification disclosing a reliable method for making the invention and showing that it operates as intended. The Examiner states that Example 5 shows experimentation that is outside the realm of routine experimentation to identify 6 microorganisms that are superior glucosamine producers. Applicants submit that first, this is an incorrect summary of Example 5 and second, that the experiment described in Example 5 is very much within the realm of routine experimentation for those of skill in the art, as will be evidenced below. Example 5 clearly shows that in a **single experiment, 96 (ninety six)** microorganisms were identified as being producers of excess glucosamine, as compared to the parent strain (thus meeting the limitations of

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the independent claims). On this point, where the Examiner seems to recognize only 6 strains as falling within the scope of the claims, Applicants emphasize that all 96 strains identified in Example 5 meet the limitations of the independent claims of having increased glucosamine-6-phosphate activity. Therefore, Example 5 shows that, in one experiment, 96 microorganisms meeting the limitations of the independent claims were identified.

The specification goes on to teach that, of these 96 microorganisms, 30 (approximately one third of the total) were identified as being superior glucosamine producers (*e.g.*, exceptional producers within the set of claimed organisms). Of these 30, 6 were selected for further study (*i.e.*, only 6 of the 30 were selected, but all 30 or even all 96 could have been selected). Of these 6, 50% (3/6) of the strains, in addition to being producers of excess glucosamine and thus meeting the independent claim limitations as described above for the entire group of 96 strains, also had the expected phenotype of reduced feedback inhibition as compared to the wild type strain, which meets the limitations of dependent Claim 56. Paralleling the fact pattern in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)), the proper viewing of the data is that only 6 of the 30 were *selected* to be screened for the additional property of reduced feedback inhibition, and of these, a respectable 50% fall within the dependent claim. Therefore, the data presented in Examples 5-6 demonstrate that it is not required that any particular amino acid be targeted for mutation in order to produce a microorganism meeting the claim limitations of the independent claim or the additional embodiment of Claim 56. As previously discussed, if one wishes to determine the identity of the mutation after the microorganism is identified by sequencing, this is a routine task. However, such knowledge is not necessary to make and use the claimed invention. Applicants again submit that the facts in the present application mirror the facts in In re Wands, *supra*, as discussed in detail in the last response.

Moreover, as further evidence that the present specification fully enables the presently claimed invention, Applicants provide herewith a Declaration under 37 CFR 1.132 of Dr. Arnold Demain, who provides an independent, third party, expert opinion on this issue. As will be seen from the Declaration and attachments, Dr. Demain is a leading expert in the field of fermentation biology and strain improvement, who has worked in this field for more than 50 years. The

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Declaration of Dr. Demain confirms that: “(1) one skilled in the art would not have to know the specific genetic modification(s) to perform on a specific gene or promoter or protein or enzyme to achieve an improved process; (2) routine experimentation in the art includes screening and/or selection; (3) such screening/selection is a necessary part of all strain improvement efforts, even when using the most modern genetic methods to cause mutations in the microbe; and (4) such experimentation is not undue or unpredictable, but is simple, rapid, and will succeed provided that the method of screening/selection is clearly stated, as it has been by the Applicants.” Dr. Demain agrees that it would not require trial and error experimentation to make and use the claimed invention, but rather, that the specification provides a carefully detailed procedure which yields a desired result. If the Examiner intends to maintain the position that Applicants or Dr. Demain are incorrect with respect to the ability to reliably and predictably produce the recited microorganisms without undue experimentation, even in view of the abundance of evidence provided to the contrary, then Applicants respectfully request that the Examiner provide documentary evidence rebutting these points or, if the Examiner is relying on personal knowledge to maintain this position, then Applicants respectfully request that the Examiner provide this statement in the form of a Declaration, pursuant to 37 CFR 1.104(d)(2).

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 40-76 under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 40-76 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has rejected Claims 40-76 under 35 U.S.C. § 112, first paragraph, on the basis of written description. Specifically, the Examiner contends that Applicants must (1) fully describe at least one species of the claimed genus sufficient to represent the genus such that a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the genus; and (2) identify the common characteristics of the claimed molecule, *e.g.*, structural, physical, and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between structure and function, or a combination of these. The Examiner asserts that the claimed invention includes many microorganisms, many glucosamine-6-phosphate synthases, and

many genetic modifications. The Examiner contends that the Declaration of Dr. Deng shows that glucosamine-6-phosphate synthases from other biological sources have low homology to *E. coli* glucosamine-6-phosphate synthase, and that the references cited by Applicants do not show that the *E. coli* glucosamine-6-phosphate synthase is representative of the claimed genus.

Applicants traverse the rejection of Claims 40-76 under 35 U.S.C. § 112, first paragraph. Initially, Applicants emphasize portions of the statements made by the Examiner. Specifically, the Examiner states that Applicants must (1) fully describe at least one species of the claimed genus sufficient to represent the genus such that a skilled artisan, in view of the prior art (emphasis added), could predict the structure of other species encompassed by the genus; and (2) identify the common characteristics of the claimed molecule, *e.g.*, structural, physical, and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between structure and function (emphasis added), or a combination of these. Applicants note that the written description requirements rely in part on what was known in the art at the time of the invention. In contrast, the Examiner seems to take the position that the entire responsibility for describing structures that were well-known in the art (*i.e.*, the sequences of other glucosamine-6-phosphate synthases) must be again described by Applicants' specification.

More particularly, the Examiner contends that the skilled artisan would not be able to predict the structure of other species encompassed by the claimed invention by the single description of the *E. coli* glucosamine-6-phosphate synthase, noting that other glucosamine-6-phosphate synthases that Applicants have demonstrated are operable in the claimed method have low overall sequence homology. Initially, Applicants respectfully remind the Examiner that sequences that have been further demonstrated to be operable in the claimed invention and those compared in the references cited by Applicants were known at the time of the present invention. Therefore, clearly, not only could one of skill in the art predict the structure of other species encompassed by the invention - indeed, one of skill in the art did not have to predict the structure of multiple other species, as they were already known in the art. As discussed previously, it was known in the art that there is a significant level of structural homology between the glucosamine-6-phosphate synthases from different organisms that relates to function.

The Examiner dismisses the publications provided by Applicants, stating that these references “do not teach that the amino acid sequence of glucosamine-6-phosphate synthase from *E. coli* is representative of the claimed genus nor do they teach how a skilled artisan can predict the structure of other species encompassed by the claimed genus from the *E. coli* glucosamine-6-phosphate synthase.” Applicants fail to understand how the Examiner can make this statement when, for example, Fernandez-Herrero et al. (1995, *Mol. Microbiol.* 17(1):1-12) compares the sequences of glucosamine-6-phosphate synthases of *E. coli*, *Rhizobium leguminosarum*, *Rhizobium meliloti*, *Thermus thermophilus*, yeast, human and mouse, a set of very divergent species, and specifically shows that all of the enzymes contain key amino acid residues that are implicated as being mechanistically important for catalytic activity. Therefore, it is clear that at the time of the present invention, the skilled artisan already knew the structural features of glucosamine-6-phosphate synthase that were correlated with function. Applicants have provided three additional references that further illustrate this knowledge at the time of the invention. The Examiner’s statement is therefore completely contradictory to what the references show, yet the Examiner has failed to provide any evidence to support his position.

Moreover, the fact that the compared sequences have low overall sequence identity (but highly conserved identity with respect to residues and regions known to be important for function), only further demonstrates that one of skill in the art would readily be able to predict other sequences that would be operable in the invention. Specifically, the fact that the enzymes have lower overall identities but nonetheless are operable is simply further evidence that the enzymes can tolerate significant modification if the structures that contribute to enzymatic function (which were known in the art at the time of the invention) are maintained. In the last response, Applicants provided a detailed discussion and publication showing that amino acid substitutions can be observed in nature in nearly one-half (50%) of the positions within an amino acid sequence while maintaining the enzymatic activity of the enzyme. Accordingly, given the guidance provided by the specification, one of skill in the art can readily predict and indeed, can select from a wide repertoire of available sequences, sequences for use in the present invention.

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Finally, Applicants submit that the United States Court of Appeals for the Federal Circuit has recently held that when the prior art "includes the nucleotide information, precedent does not set a *per se* rule that the information must be determined afresh", and that it is not correct to conclude that "§112 imposes a *pre se* rule requiring recitation in the specification of the nucleotide sequence of the claimed DNA, when that sequence is already known in the field". *Capon et al. v. Eshhar et al. v. Dudas*, Nos. 03-1480 and -1481 (Fed. Cir. Aug. 12, 2005). In the present specification, Applicants have specifically exemplified in detail the use of a recombinant nucleic acid molecule encoding an *E. coli* glucosamine-6-phosphate synthase, and have taught that the invention is not limited to the *E. coli* glucosamine-6-phosphate synthase and that the methodology is applicable to synthases from other microorganisms, since other microorganisms have similar amino sugar metabolic pathways and genes and proteins having similar structure and function within such pathways. Moreover, Applicants have demonstrated by evidence provided earlier to the Examiner that indeed, many other glucosamine-6-phosphate synthases were known in the art at the time of the invention.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 40-76 under 35 U.S.C. § 112, first paragraph.

Applicants have attempted to address all of the Examiner's concerns as set forth in the April 21, 2005 Office Action and submit that the claims are in a condition for allowance. Any further questions or concerns regarding Applicants' position should be addressed to the below-named agent at (303) 863-9700.

Respectfully submitted,

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